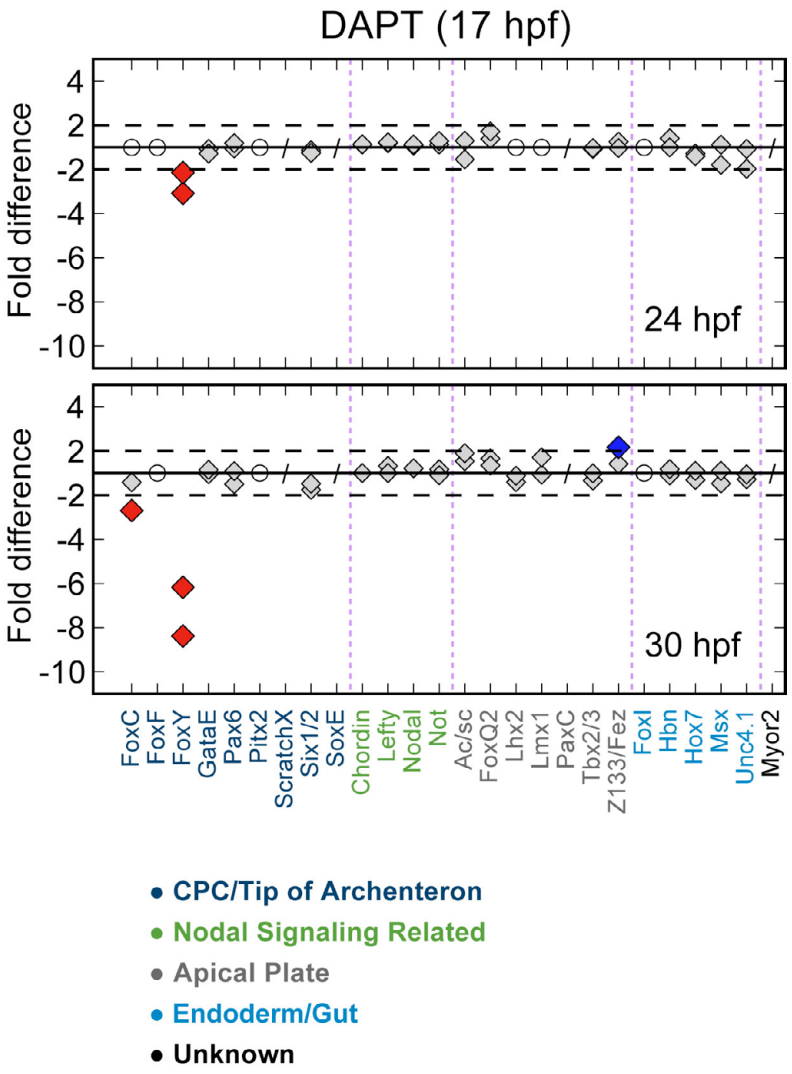

Fig. S1. Effects of Delta/Notch perturbations on transcript levels at 24 hpf and 30 hpf for the same selection of genes as shown in Fig. 2. DAPT treatment at 17 hpf causes strong and consistent depletion of only *foxY* among the 182 genes included in this dataset. The data are reformatted from Materna and Davidson (Materna and Davidson, 2012). Each diamond represents a single experiment; red indicates a significant reduction, blue an increase, and gray no significant change of transcript levels following treatment. Genes not significantly transcribed in experiment and control are marked with an open circle. We estimated transcript levels by comparing our data with prior absolute measurements (Materna et al., 2010). A level of 25 transcripts/embryo was chosen as significant expression. Genes not tested are marked with a solidus. Black dashed lines indicate the threshold for significant changes following perturbation.

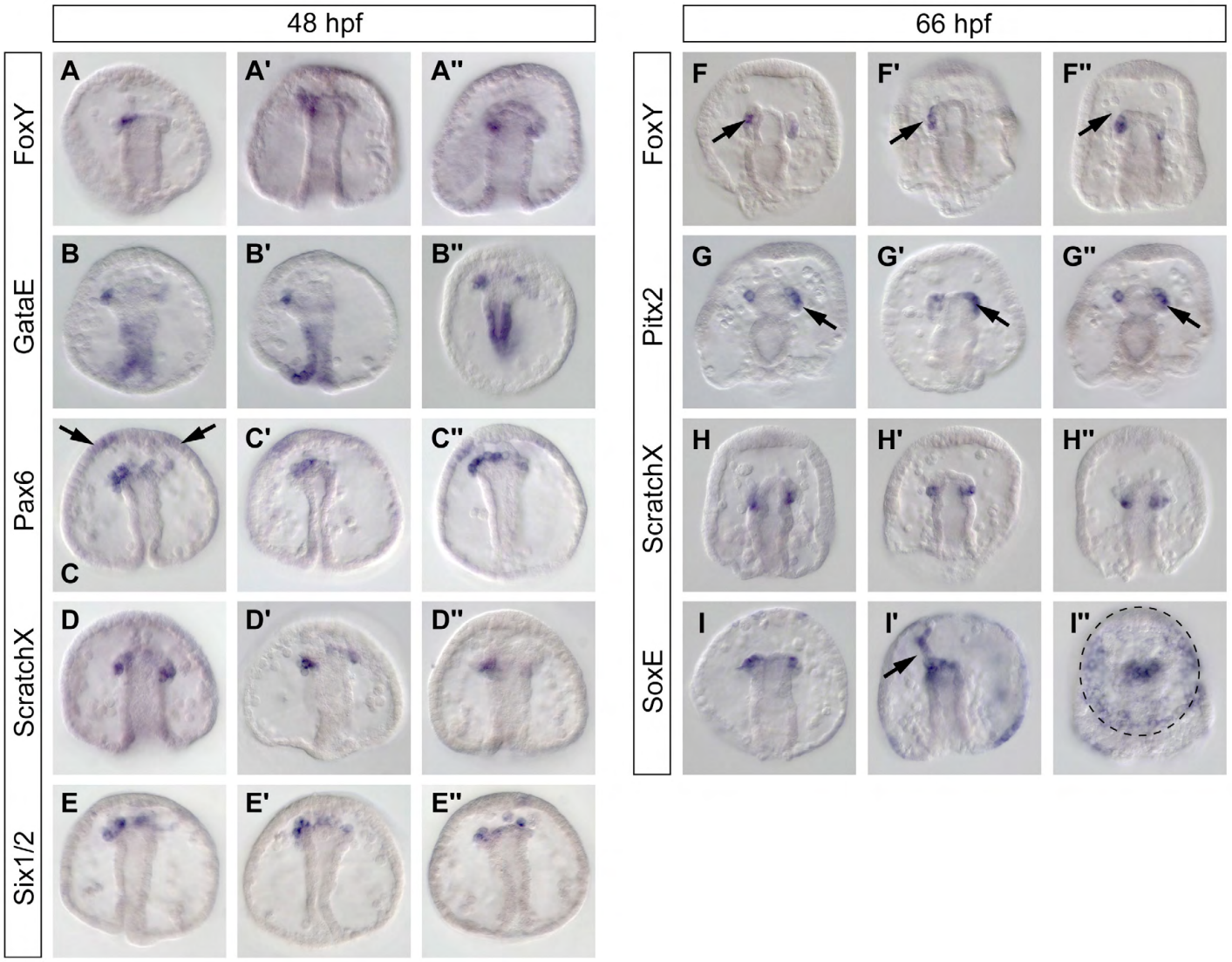

Fig. S2. Additional spatial expression patterns of genes with a specific coelomic pouch expression compartment. (**A-A″**,**F-F″**) *foxY* expression is biased to the left side at 48 hpf, but becomes restricted to the left side after 66 hpf (arrows in F-F″). (**B-B″**) *gataE* is expressed in the gut and the coelomic pouches, and is biased to the left side. (**C-C″**) *pax6* is expressed at the tip of the archenteron, but also in the ectoderm surrounding the apical plate (arrows in C). (**D-D″**,**H-H″**) *scratchX* is expressed in both pouches and remains bilaterally expressed until at least 3 dpf. (**E-E″**) At 48 hpf *six1/2* is expressed at the tip of the archenteron. (**G-G″**) *pitx2* is preferentially expressed in the right coelomic pouch (arrow). *pitx2* and *foxY* expression patterns are complementary; overlap is minimal at best (compare G-G″ with F-F″). (**I-I″**) *soxE* is expressed in the coelomic pouches and the water canal as it emerges from the left coelomic pouch (arrow in I′). It has a second expression domain in the ciliated band (dashed line in I″).

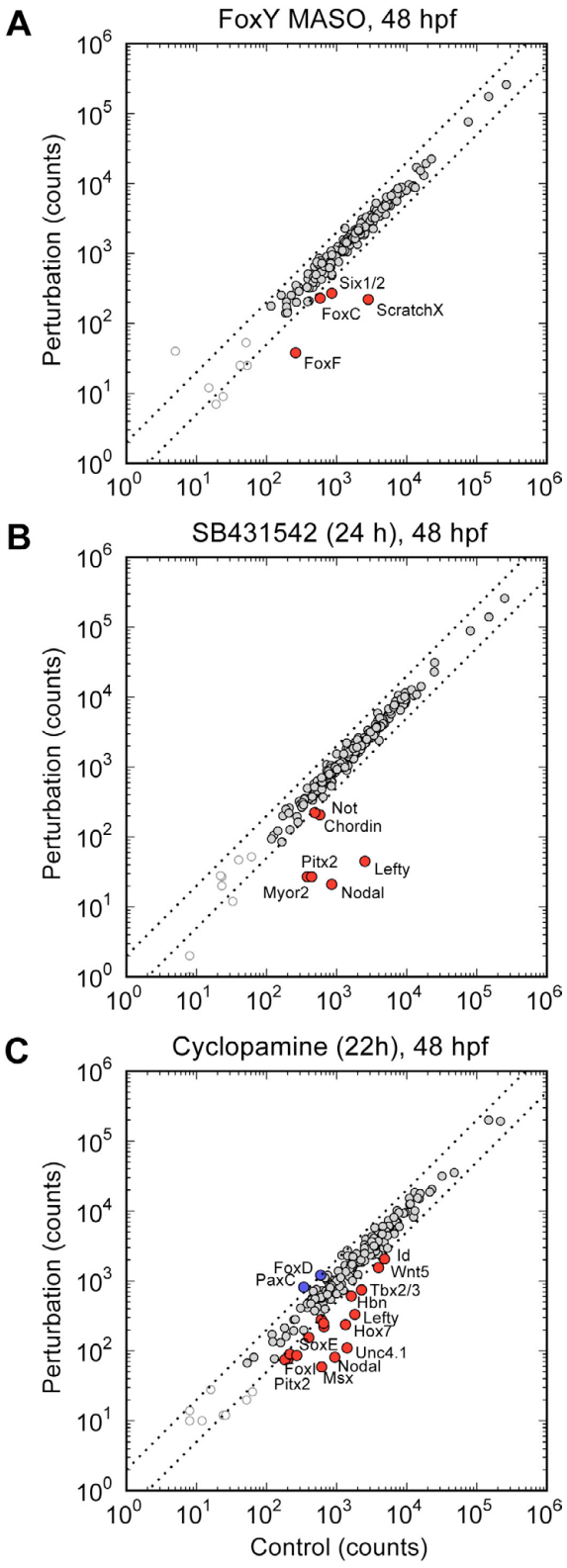

Fig. S3. Global evaluation of perturbation effects by transcript quantification. RNA from treated embryos was extracted and quantified using the NanoString nCounter and the resulting counts were normalized. Counts from perturbed embryos were plotted against those of control embryos. (**A**) FoxY MASO injection at fertilization causes depletion of transcript levels for genes specifically expressed in the coelomic pouch progenitors. Out of 205 genes only a few, none of them outside the *foxY* expression compartment, are affected by the perturbation, indicating that the MASO does not cause nonspecific delays in development. (**B**) Nodal perturbation by addition of the inhibitor SB431542 at 24 hpf causes lower transcript levels of only six genes included in the NanoString code set. (**C**) Disruption of Hh by application of cyclopamine has more widespread effects and also causes depletion of several endoderm genes. Labels for some weakly affected genes that were not substantiated in repeat experiments were left out of C for clarity. The dotted lines indicate a threshold of 2-fold change. A red dot indicates significant depletion following treatment, a blue dot a significant increase, and a gray dot indicates no significant change. Genes present with ~25 transcripts or fewer per embryo are marked with an open circle; transcription levels were estimated from prior absolute measurements (Materna et al., 2010).

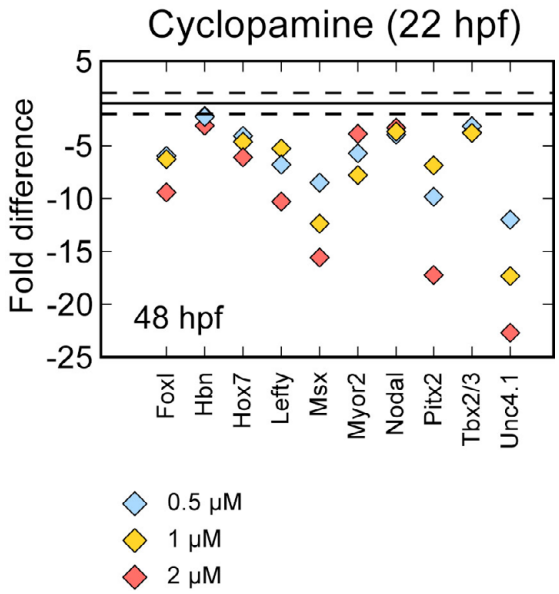

Fig. S4. Effects of cyclopamine treatment are dose dependent. Sea urchin embryos from the same batch were treated with the indicated concentrations of cyclopamine to disrupt Hh signaling. RNA was extracted and quantified with the NanoString nCounter and fold differences were calculated between controls (ethanol only) and cyclopamine-treated embryos. This plot shows the genes that are most strongly affected by the perturbation (compare with Fig. 4B and Fig. S3C). In our experiments, cyclopamine concentrations of 5 µM and higher caused severe developmental delays and death.

FoxY Construct #3 upstream region (-778 to ATG/GFP in second exon)

TTCGCAGGCTAGAGACACCTCTCTGCTTAATCCGGAAGCTTAAACAAAGAGCCCTCATTGTCAATGGTGTAGTG
TACGAGGCGGATTAATAAATCCAATTAGAAGGGTATCACCAGGCGTGGGAAAAGGAATGGGTCTGACTTGGCTA
GTCTTATCATTTCATCATCAGACCGTTTTGAAAACAATCCATAATAGATTAGAGTCAATAAATTAGACAGATGA
TAAAATGGGTCTTGATTTGCTATAGATCGTTGGCATACGCCATGCATTGGGTATGATGTCTTTTAAGCCATTCG
AACAAAACACTATATATGCAATCATGCACAATCTGATTTCTTCTTCTCTGTCTTTCTCTCCATGCCCCCTCCCC
GCCCCCCCCCCCCTCTCTCTCTCCTCTATCTCAGAGAGTTTCTGTCTCTATATAGAAACTCTCTAGACATTA
TTTGAAACTTCTATGATTGTACAATATGCGTTTGGACAATTGTCATGTTTGTGAATTTTGAGTATCATTTTTAT
ATGAGAGTGATATTTTGTTGATCGACGGTATGATTGAAAAAGATCAACTTGGTCTGATTTCAAAGACTACCATA
TAGAAAAAGATTGTTCGCGCATGCGTCGCCCTGATCCTTTCTTTCGCATCTCGATTACGTAACACCTCGCTCTC
CTGTAATTGGCTTAGAGTTGGTAAGAACGGTGAGAACAGATCAGGTATAAAGTAAGATAGAAGTCCGATGAAGA
AGCTTATCTTGCTCAAGACCTCTGAACTGGAAACATTGCTGCACTGACTCTGCCTACATACCCAGCTTCACAAA
TCTCGCCTCATCGATTGCCTTACTCCTCATTTTCCATACCATTTGTAGGTGAGTATCTCACGTTAAATTGTAAT
ATTATCTGTCGTAATAATGATTTGACTCCATTTCTGTAGTACTCGTGGTGATTTAAGTGTTTGGAGCGAATGTT
AGTCTTGTTGGTTGAATGGTGACTTGCGTTTGTTTTCTTATCGATGAGAGGGGAATTCGTGTGAAGGGTTTTTA
AATTACAGATGCCAATATTCCAATATCAGTTCAGCTTAACATCCAGTAAAACCAGTAATATACAATATACAAAC
ATATCTCATCAATTTCTTTAATATGTTCAATTAGGCATGTAAATTACTCAGGGAACATCTATAGTATATAGGCC
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ATGATCAGGTATATTTTTGTTCTGTTATACGTGAGCTTTGAGTGTCGTACATAATATATGTATATTCTTTGTAA
ATATTTTTGCGTGGAATTCGTTTGCCCCAAAATGATTATATTAGTGTTGGTTTTATGAATGTCTATACAATCGA
TACCTTTTTATGATTGTTATTCATGAATCAAAGATGAAATAAAGAAGGAAAAAATGAACCCGTTGTTTTCCGGA
GATGTGGGAGGTAAATTTATGACCAAAAATCTACTTGTCTTTTTTTCACTTTTCATGAATAGTGTTCTGCACTT
GGAGCCATGAGCAAGGGCGAGGAACTGTTCACTGGCG...

- Pitx2 binding sites
- Start of Construct #4
- Su(H) binding sites
- Exon
- Splice Site
- Start codon
- GFP sequence

Fig. S5. Annotated sequence of the regulatory region upstream of the *foxY* transcription start site. This sequence corresponds to the region contained in Construct 3; Construct 4 is nested within, starting at position 379 (marked in blue). 5′-(C/T)(A/G)TG(A/G)GA(A/G/T)-3′ was used to identify candidate Su(H) sites (Ransick and Davidson, 2006). The TG at positions 3 and 4, and the GA at positions 6 and 7, were mutated to CA and AG. We used the sequence 5′-GGATTA-3′ to identify candidate Pitx2 target sites, and mutated 5′-GATT-3′ to 5′-AGCC-3′.

Table S1. Primers for WMISH templates and QPCR

Gene	Forward primer	Reverse primer	Use	Accession No.
eya	GTATTGGAAGAGGGCGTCAA	AGGAGGGGTGGATAGCATCT	WMISH template	SPU_013869
foxY	ACTGGATGATCAACCCAAGC	AGAAACCTTTGGTGGTGTCG	WMISH template	AF517552.1
foxY	TGCACTGCACTGACTCTGC	CTTTCCATTCCGTGGTGAAG	QPCR	AF517552.1
gataC	GCTGGAGTAGCAAGCAGTCAAG	TGTTACCCATTACCCCAGGATG	WMISH template	NM_214539.1
gataE	TCTTACCCATCAGCCAGGAC	TCGAGTGGAACAATGGAACA	WMISH template	NM_001005725.1
hmg1	GGACCGAGACAGTTCAAAGC	CTTCTCCTCCAGAGCCTTCC	QPCR	SPU_027981
lefty	GAACTTCATACGACAAACATCTCCTGGC	TTTCGATTATATCATATTATTGCATTTTATTATG	WMISH template	SPU_009911
nanos	GCAAGAACAACGGAGAGAGC	CCGCATAATGGACAGGTGTA	QPCR	DQ286228.1
nodal	CACAAAGTGTGTTTGTGCAAG	GTCGATGAAATTGAAAATATCATGA	WMISH template	NM_001098449.1
not	ACGATTTGGAGGGTTTTAAGC	GCAACTTACCCGTCATCACC	WMISH template	NM_214562.1
pax6	GACAGTGTTCTCATCGTGGTG	ACCTCGCGAGAGAAAATCAA	WMISH template	SPU_006786
pitx2	ATCCCAAATTTCCCGTCTTC	TTGGTGGTGTCTCATGTGGT	WMISH template	SPU_004559
scratchX	CGGCCTAACAAGGAGAAAGC	ACGGTTTAATGGTGGAGTGC	WMISH template	SPU_15640
six1/2	TGAAACACCGTCAAAACAAGG	AACTTGTGTCGTGGTCAGTCC	WMISH template	SPU_017379
soxE	GGAGTAGCCCAAGAACACCA	TGACTGTACCTGCGAAGGAG	WMISH template	SPU_016881
ubq	CACAGGCAAGACCATCACAC	GAGAGAGTGCGACCATCCTC	QPCR	SPU_021496